ADDITIONAL INFORMATION

Supplemental Tables

Statistics data from this study

Table S1a. Statistics Data (Related to Figure 2)

	Cell line	(Mean ± SEM)(%)
SOX9 (ICC)	FTD3 patient 1	91,75±0,8764
	Isogenic control 1	92,57±0,6373
	FTD3 patient 2	86,69±2,394
	Isogenic control 2	93,33±4,216
	Homozygous	88,14±4,707
	Heterozygous	88,21±1,267
	Wildtype	91,33±2,657
AQP4 (ICC)	FTD3 patient 1	88,67±6,418
	Isogenic control 1	94,73±0,1618
	FTD3 patient 2	94,07±3,229
	Isogenic control 2	97,5±2,5
	Homozygous	96,43±3,573
	Heterozygous	95,00±0,00
	Wildtype	100,00±0,00
S100β (ICC)	FTD3 patient 1	75,03±3,746
	Isogenic control 1	86,22±4,288
	FTD3 patient 2	86,74±2,649
	Isogenic control 2	97,22±2,778
	Homozygous	91,03±3,179
	Heterozygous	100,00±0,00
	Wildtype	100,00±0,00

Significant differences are indicated by *p < 0.05.

Table S1b. Statistics Data (Related to Figure 3)

	Cell line	Control (Mean ± SEM)	200nM rapamycin (Mean ± SEM)	500nM rapamycin (Mean ± SEM)
P62 average area of distribution (Based on ICC)	FTD3 patient 1	14,4±2,668*	20,7±3,62**	20,57±3,41**
	Isogenic control 1	7,25±1,11	4,74±0,43	4,74±0,27
	FTD3 patient 2	10,21±1,714**	8,54±1,09**	5,52±0,74*
	Isogenic control 2	2,53±0,08	4,08±0,27	3,71±0,14
	Homozygous	74,06±10,26**	82,18±7,82**	63,45±5,46**
	Heterozygous	28,73±3,59	16,47±2,24**	13,77±1,75**
	Wildtype	18,92±6,67	9,93±1,95	9,77±1,74
LC3B (Based on WB)	FTD3 patient (pooled)	0,1005±0,0034****	Na	Na
	Isogenic control (pooled)	0,423±0,0339	Na	Na
	Introduced mutations (pooled)	0,201±0,0137***	Na	Na
	Wildtype	0,945±0,2107	Na	Na
Colocalization of P62 and RAB7	FTD3 patient 1	482,3±71,26**	949,5±109,1**	725,4±97,29**
	Isogenic control 1	38,6±8,48	78,8±14,98	69,8±18,19
	FTD3 patient 2	471,2±86,63**	530,6±77,07**	313,8±84,61**
	Isogenic control 2	36,1±8,4	134,3±36,41	59,2±10,31
	Homozygous	1227±222,4**	1660±163**	1190±114,1**
	Heterozygous	1199±150,9**	1283±157,6**	1395±165,4**
	Wildtype	310,4±50,9	355,9±59,6	381,9±56,19
Colocalization of P62 and LAMP1	FTD3 patient 1	368,7±40,2**	321,3±32,83**	273,1±31,3**
	Isogenic control 1	81,8±35,81	67±30,66	8,3±1,95
	FTD3 patient 2	637,2±128**	732±106,5*	848,6±113,2**
	Isogenic control 2	192,7±60,59	376,7±75,58	227,1±34,46
	Homozygous	588,9±140,5	413,3±87,32	244,6±64,41**
	Heterozygous	761,2±126,4	978,4±143,4**	885,5±106
	Wildtype	497,5±99,51	512,8±70,41	675,6±102,5
LAMP1 (Based on WB)	FTD3 patient (pooled)	2,596±0,0286	Na	Na
	Isogenic control (pooled)	0,938±0,1166	Na	Na

	Introduced mutations (pooled)	2,62±0,7938	Na	Na
	Wildtype	0,506±0,4918	Na	Na
P62 (Mouse)	Control mouse	1,000±0,0306	Na	Na
	CHMP2B mouse	1,487±0,183	Na	Na
Rab7 (Mouse)	Control mouse	1,000±0,0103	Na	Na
	CHMP2B mouse	1,323±0,1200	Na	Na
Lamp1 (Mouse)	Control mouse	1,000±0,0474		
	CHMP2B mouse	1,338±0,1477		

Significant differences are indicated by *p < 0.05, **p <0.01 and ***p <0.001.

Table S1c. Statistics Data (Related to Figure 4)

	Cell line	(Mean ± SEM)
ROS (Mean Fluorescence Intensity)	Homozygous	87332±847*
	Wildtype	78406±1994
Mitotracker (Running average length (µm))	FTD3 patient 1	42,56±1,605**
	Isogenic control 1	28,46±0,8091
	FTD3 patient 2	47,91±2,601**
	Isogenic control 2	33,2±1,016
	Homozygous	66,05±2,44**
	Heterozygous	42,51±2,008**
	Wildtype	39,28±1,351
RT-qPCR (*OPA1*)	Homozygous	1,37±0,109
	Heterozygous	2,15±0,173*
	Wildtype	1±0,294
RT-qPCR (*MFN1*)	Homozygous	3,32±0,252*
	Heterozygous	1,219±0,075
	Wildtype	1±0,032
RT-qPCR (*FIS1*)	Homozygous	14,21±0,751*
	Heterozygous	6,37±0,828*
	Wildtype	1±0,150
RT-qPCR (m*OPA1*)	Control mouse	0,83±0,114
	CHMP2B mouse	1,09±0,072
RT-qPCR (m*MFN1*)	Control mouse	0,189±0,042
	CHMP2B mouse	0,39±0,0931
RT-qPCR (m*FIS1*)	Control mouse	0,165±0,002
	CHMP2B mouse	0,181±0,0074

Significant differences are indicated by *p < 0.05 and **p <0.01.

Table S1d. Statistics Data (Relative to Figure 5)

Metabolic Labeling	Cell line	(Mean±SEM)
Lactate	FTD3 patient 1	14,0±1,4****
	Isogenic control 1	29,2±1,74
	FTD3 patient 2	11,3±3,4****
	Isogenic control 2	51,6±2,7
	Homozygous	21,6±4,2*
	Heterozygous	24,8±5,0*
	Wildtype	47,3±4,4
Alanine	FTD3 patient 1	5,3±1,7***
	Isogenic control 1	15,4±0,3
	FTD3 patient 2	9,6±3,1
	Isogenic control 2	14,3±0,1
	Homozygous	15,4±2,9**
	Heterozygous	11,5±0,6*
	Wildtype	27,3±2,9

Significant differences are indicated by *p < 0.05, **p <0.01, ***p <0.001 and ****p <0.0001.

Table S1e. Statistics Data (Related to Figure 5)

	Cell line	(Mean±SEM)
LCN2	FTD3 patient 1	1666±523,5**
	Isogenic control 1	96,8±16,54
	FTD3 patient 2	856,1±316,7*
	Isogenic control 2	92±22,74
	Homozygous	1405±345,9**
	Heterozygous	1613±548,7*
	Wildtype	89,38±46,87
C3 (Based on WB)	FTD3 patient (pooled)	0,0095±0,0016*
	Isogenic control (pooled)	0,0046±0,0004
	Introduced mutations (pooled)	0,0793±0,0178
	Wildtype	0,0384±0,0281
Ratio of p-Nf-Kb/Nf-KB (Based on WB)	FTD3 patient (pooled)	0,073±0,0737
	Isogenic control (pooled)	0,0457±0,004
	Introduced mutations (pooled)	2,393±1,327
	Wildtype	1,191±1,151
Cytokine Analysis (IL-6)	FTD3 patient 1	3721,64±173,89**
	Isogenic control 1	571,71±59,17
	FTD3 patient 2	3006,64± 78,80**
	Isogenic control 2	134,72±7,69
	Heterozygous	1099,92±4,63**
	Wildtype	26,87±0,183
Cytokine Analysis (IL-8)	FTD3 patient 1	707,15±12,10*
	Isogenic control 1	393,87±52,75
	FTD3 patient 2	769,511±11,532**
	Isogenic control 2	64,59±1,23
	Heterozygous	348,16±3,31**
	Wildtype	44,65±0,865
Cytokine Analysis (IL-13)	FTD3 patient 1	79,90±1,69**
	Isogenic control 1	25,63±0,419
	FTD3 patient 2	42,29±12,17*
	Isogenic control 2	4,48±1,55
	Heterozygous	22,71±0,77*
	Wildtype	3,22±0,434
Neurite Length	FTD3 patient (Pooled)	6951±913
	Isogenic Control (Pooled)	1,054e+007**
MitoSox (mROS) Fluorescence Percentage	Homozygous (Before Treatment)	100%±2.5%
	Homozygous (After UDCA Treatment)	64.32±1.70% *
RT-qPCR (m*c3*)	Control mouse	1,00±0,002
	CHMP2B mouse	2,42±0,566
RT-qPCR (m*Lcn2*)	Control mouse	1,00±0,01
	CHMP2B mouse	1,55±0,791
RT-qPCR (m*Il6*)	Control mouse	1,00±0,0004
	CHMP2B mouse	2,018±0,923

WB (mc3)	Control mouse	0,116±0,036
	CHMP2B mouse	0,215±0,0098
WB (mGFAP)	Control mouse	0,19±0,103
	CHMP2B mouse	0,095±0,007

Significant differences are indicated by *p < 0.05 and **p <0.01.

Table S1f. Statistics Data (Related to Figure S1, S2, S3)

	Cell line	Control (Mean ± SEM)	200nM rapamycin (Mean ± SEM)	500nM rapamycin (Mean± SEM)
LC3B	FTD3 patient 1	297,4±35,85**	209,7±17,61	272,8±34,01*
	Isogenic control 1	139,4±24,01	202,4±17,07	147±29,24
	FTD3 patient 2	301±47,25**	419,1±60,76**	582,8±76,06**
	Isogenic control 2	31,2±4,70	55,5±12,26	95±18,02
	Homozygous	1758±333,8**	2643±432**	2393±279**
	Heterozygous	609,3±166,2*	543,3±39,86*	386,9±70,33*
	Wildtype	223±70,68	266,3±97,67	183,8±37,99
P62 puncta numbers	FTD3 patient 1	2685±357,5**	2877±351,7**	2220±223,4**
	Isogenic control 1	349,5±45,21	528,5±55,91	387,9±87,43
	FTD3 patient 2	2196±278,8**	2234±200,4**	1818±278,8**
	Isogenic control 2	913,1±191,2	840±170,7	725,7±164,8
	Homozygous	1138±136,4	1279±152,7	1333±169,2
	Heterozygous	2606±311,5**	3071±350,2**	3361±218,6**
	Wildtype	1065±248,6	1144±201,2	1223±142,4
RAB7 puncta numbers	FTD3 patient 1	961,1±118,4**	1723±174,4**	1272±124,6**
	Isogenic control 1	183,1±51,86	399,2±70,19	536,8±120,2
	FTD3 patient 2	1449±285,7*	1440±180,1	1243±312
	Isogenic control 2	729,3±144,1	997,1±184,3	549,7±123,8
	Homozygous	2023±366,5**	2279±201,4*	1848±200,1
	Heterozygous	2164±243,4**	2279±332,5**	2951±288**
	Wildtype	882,8±123,2	1290±312,9	1303±175,4
LAMP1 puncta numbers	FTD3 patient 1	692,6±122**	563,2±74,6**	596,7±60,51**
	Isogenic control 1	95,4±19,98	200,1±73,82	137±43,23
	FTD3 patient 2	1242±207,5**	1190±229,8**	965,3±166,6**
	Isogenic control 2	392±41,2	253,6±25,73	235±26,84
	Homozygous	878,5±174,5	1021±211,7	553±135,6**
	Heterozygous	1279±251,6	1381±153,5	1608±208,8
	Wildtype	1315±307	1528±250,1	1909±347,2

Significant differences are indicated by *p < 0.05, **p <0.01 and ***p <0.001.

Table S1g. Statistics Data (Related to Figure S6)

	Cell line	Control (Mean ± SEM)
Cytokine Analysis (TNFα)	FTD3 patient 1	9,49±1,82
	Isogenic control 1	7,33±0,639
	FTD3 patient 2	10,04±2,26*
	Isogenic control 2	0,45±0,003
	Heterozygous	3,89±0,475
	Wildtype	0,535±0,074
Cytokine Analysis (IL-2)	FTD3 patient 1	9,54±2,99
	Isogenic control 1	2,003±0,62
	FTD3 patient 2	6,17±1,24
	Isogenic control 2	1,00±0,18
	Heterozygous	5,26±0,76
	Wildtype	1,1±0,04
Cytokine Analysis (IL-β)	FTD3 patient 1	5,94±0,162
	FTD3 patient 2	6,373±0,154
	Heterozygous	2,05±0,082

Significant differences are indicated by *p < 0.05.

Table S2

Subset of significantly differentially expressed genes detected by comparing patient-derived CHMP2B-mutant cell lines with Cas9-corrected controls. For a full list of significantly DE genes, see Table s3. Genes are grouped based on their association with processes, functions or characteristics of interest for the disease. Lists of genes related to each annotation were retrieved from one of the following sources as indicated: Gene Ontology (GO); WikiPathways; COMPARTMENTS; KEGG: literature (manually curated). For each gene, we report its name, Ensembl identifier, log2 fold change and adjusted P-value. Only significantly differentially expressed genes are reported (adjusted p-value <= 0.05, absolute log2 fold change >=1 and average of normalized read counts >=10). For genes grouped by associated compartment, we also provide the confidence score assigned in COMPARTMENTS; only genes with a confidence score >=3 is shown.

Table S3

List of significantly differentially expressed genes detected by comparing patient-derived CHMP2B-mutant cell lines with Cas9-corrected controls, and overrepresented terms obtained by comparing differentially expressed genes with expressed genes. For each differentially expressed gene, we report its name, Ensembl identifier, log2 fold change and adjusted P-value. Gene counts normalized by DeSeq2 in the sample are also provided. Only significantly differentially expressed genes are reported (adjusted p-value <= 0.05, absolute log2 fold change >=1 and average of normalized read counts >=10). Overrepresented terms were filtered by excluding redundant annotations (overlap between gene sets >= 50%).

Table S4. Antibodies used for Immunocytochemistry

	Antibody	**Antibody Registry* Identifier**	**Dilution**	**Company**
Astroglial differentiation	rabbit anti-SOX9	AB_2665492	1:400	CST
	rabbit anti-GFAP	AB_10013382	1:1000	Dako
	rabbit anti-aquaporin4	AB_2274338	1:50	Abcam
	mouse anti- S100β	AB_882426	1:500	Sigma
Reactive Astrocyte	rabbit anti-LCN2	AB_10618739	1:200	Millipore
	mouse anti-TIA1	AB_2201439	1:100	Abcam
	rabbit anti-C3	AB_1240642	1:500	GeneTex
Autophagy-Endolysosomal pathway	rabbit anti-LC3B	AB_881433	1:1000	Abcam
	rabbit anti-LC3B	AB_881429	1:2000	Abcam
	mouse anti-RAB7	AB_882241	1:1000	Abcam
	guinea pig anti-P62	AB_2687531	1:100	Progen
	mouse anti-LAMP1	AB_2296838	1:400	HB
Secondary antibodies	AF 488 donkey anti-rabbit IgG	AB_2534015	1:1000	TFS
	AF 488 donkey anti-guinea IgG	AB_2535788	1:2000	TFS
	AF 594 donkey anti-rabbit IgG	AB_2556547	1:2000	TFS
	AF 594 donkey anti-goat IgG	AB_2534105	1:1000	TFS

	AF 594 donkey anti-mouse IgG	AB_253578	1:1000	TFS
	AF 647 donkey anti-mouse IgG	AB_162542	1:1000	TFS

AF, Alexa Fluor; TFS, Thermo Fisher Scientific Inc; HB, Hybridoma bank; CST, Cell Signalling Technology, http://antibodyregistry.org/

Table S5. Antibodies used for Western blot

	Antibody	Antibody Registry* Identifier	Dilution	Company
Autophagy Proteins	*rabbit anti-GAPDH	AB_9485, AB_307275	1:3000 1:3000	Abcam Abcam
	*mouse anti-GAPDH	AB_627678	1:4000	SCT
	rabbit anti-LC3B	AB_881429	1:3000	Abcam
	rabbit anti-C3	AB_1240642	1:10000	GeneTex
	mouse- LAMP1	AB_2296838	1:4000	HB
	rabbit-Phospho NF-Kb	AB_10827881	1:1000	CST
	rabbit- NF-Kb	AB_10859369	1:1000	CST
	mouse- Caspase 3	AB_781826	1:500	SCT
Secondary antibodies	IRDye® 800CW Donkey anti-Rabbit IgG (H + L)	AB_621848	1:15,000	LI-COR
	IRDye® 800CW Donkey anti-Mouse IgG (H + L)	AB_621847	1:15,000	LI-COR
	IRDye® 680LT Goat anti-Mouse IgG (H + L)	AB_10706161	1:20,000	LI-COR
	IRDye® 680LT Goat anti-Rabbit IgG (H + L)	AB_10706309	1:20,000	LI-COR

HB, Hybridoma bank; CST, Cell Signalling Technology; IRDye, Infrared Dye; http://antibodyregistry.org/; GAPDH was used as reference gene.

Table S6. Primers used for RT-qPCR

	Human Primers	
Gene Name	**Forward**	**Reverse**
OPA1	GGCGGAAGACCTCAAGAAAGT	GGCTGGACAAAAGACGTTGAT
MFN1	CCAGAAAGTGGTGTGGCACT	GTTTTCACTGCTGACTGCGAG
FIS1	GGTGCGGAGCAAGTACAATGA	CGTATTCCTTGAGCCGGTAGT
MAP3K9	CTGGAACGGGAGCTCAACAT	TGGTGTCAACTGGATGGCTC
DCN	CCCTCCTCCTTTCCACACCT	TTTTCACAACCAGGGAACCTTT
GAS6	GACATAGACGAGTGCGCAGA	ACGGCAAGATGTCCTCACAG
GAD1	GGGAACTAGCGAGAACGAGG	GGTATCGTACGTTGTGGGGC
MT-ND2	TCATAGCAGGCAGTTGAGGC	GGTCGTGGTGCTGGAGTTTA
IL17D	GAGTCCCCGGGTCTGGAT	GTGTGGTGGAAGGCACTGAG
**GAPDH*	CTCTCTGCTCCTCCTGTTCGAC	TGAGCGATGTGGCTCGGCT
	Mouse Primers	
Gene Name	**Forward**	**Reverse**
mIl6	GATGCTACCAAACTGGATATAATC	GGTCCTTAGCCACTCCTTCTGTG
mc3	ACCCCTTCATTCCTTCCACCT	CCTTACTGGCTGGAATCTTGATGG
mLcn2	TCTGTCCCCACCGACCAATG	GGGGAGTGCTGGCCAAATAA
mMap3k	ATCAGGAGATGAAGGCCTCAAG	AGGACTGGTTGGGTGAATGC
mMfn1	CAGGGACGGAGTGAGTGTCC	GTTTCTGCCATTATGCACCTGGA
mFis1	CTGGTGTCTGTGGAGGATCTGA	GAGCCTTTTCATATTCCTTGAGCC
mOpa1	CTGCAGGTCCCAAATTGGTT	CTGCAGGTCCCAAATTGGTT
mDcn	TTCCTACTCGGCTGTGAGTC	AAGTTGAATGGCAGAACGC
mGad1	CCTTCGCCTGCAACCTCCTCGAAC	GCGCAGTTTGCTCCTCCCCGTTCTT
mMt-nd2	AGGGATCCCACTGCACATAG	TGAGGGATGGGTTGTAAGGA
mIl17d	GGGCGTACAGGATTTCCTAC	AGAGAAGACGGGTGTGCTG
mGas6	AAAGGGCCAGAGTGAAGTGA	TTTTCCCGTTTACCTCCAGA

*mGapdh	TGCACCACCAACTGCTTAG	GGATGACCTTGCCC

*was used as reference gene; m=mouse.

Media used in this study

Table S7a. Neural Maturation Medium (NMM)

Reagent	Company & Catalogue	stock	Final	role
DMEM/F12 with L-Glutamine and Hepes	Thermo Fisher Scientific, 11330-057	1x	50 %	Basal medium
Neurobasal Medium	Thermo Fisher Scientific, 21103049	-	50 %	Basal medium
B-27 without Vitamin A	Thermo Fisher Scientific, 12587-010	50x	2 %	Support neuronal cell growth
N-2 Supplement	Thermo Fisher Scientific, 17502048	100x	1 %	Support neuronal cell growth
Glutamax (L-Glutamine)	Thermo Fisher Scientific, 35050061	100x	1 %	Nutrition factor
Penicillin/Streptomycin	Sigma Aldrich, P0781	100x	1 %	Antibiotics
*b-FGF-2	ProSpec, CYT-557	25ng/µl/dPBS	10 ng/ml	Promotes proliferation
*EGF	ProSpec, CYT-217	100 ng/µl	10 ng/ml	Promotes proliferation
*SB4315642	Selleckchem, S1067	10 mM/DMSO	10 µM	Inhibition of TGFβ pathway. Promotes ectodermal differentiation [1]
*LDN193189	Sigma Aldrich, SML0559	2 mM	0.1 µM	Inhibition of BMP pathway. Promotes neural differentiation [2]

Table S7b. Astrocyte Differentiation Medium (ADM)

Reagent	Company & Catalogue	Stock	Final	Role
Neurobasal Medium	Thermo Fisher Scientific, 21103049	-	90 %	Basal medium
Non-essential Amino Acids	Sigma Aldrich, M7145	100x	1 %	Amino Acid supplement
N-2 supplement	Thermo Fisher Scientific, 17502048	100x	1 %	Supplement to support neuronal cell growth
Penicillin/Streptomycin	Sigma Aldrich, P0781	100x	1 %	Antibiotics
*L-Ascorbic acid	Sigma Aldrich, A4403	50 mM	200 µM	Antioxidant
*Recombinant Human IGF-1	Peprotech, 100-11	100 µg/ml	200 ng/ml	Growth Factor
*Human Activin-A	Thermo Fisher Scientific, PHG9014	100 µg/ml	10 ng/ml	Growth Factor
*Recombinant Human Heregulinβ-1	Peprotech, 100-03	100 µg/ml	10 ng/ml	Growth Factor
*b-FGF-2	ProSpec, CYT-557	25 ng/µl	10 ng/ml	Growth factor Promotes proliferation

Table S7c. Astrocyte Maturation Medium (AMM)

Reagent	Company & Catalogue	stock	Final	ROLE
Neurobasal Medium	Thermo Fisher Scientific, 21103049	-	50 %	Basal medium
DMEM/F12	Sigma Aldrich, D8437	1:1	50 %	Basal medium
Non-essential Amino Acids	Sigma Aldrich, M7145	100x	1 %	Amino Acid supplement
N-2 Supplement	Thermo Fisher Scientific, 17502048	100x	1 %	Supplement to support neuronal cell growth
L-Ascorbic Acids	Sigma-Aldrich, A4403	50 mM	200 µM	Antioxidant
Glutamax (L-Glutamine)	Thermo Fisher Scientific, 35050061	100x	1 %	Nutrition Factor
Sodium Pyruvate	Thermo Fisher Scientific, 1136070	100 mM	1 %	Nutrition Factor Carbon source
Fetal Bovine Serum	Th Geyer, BW/S181B-500	100x	2 %	Basal media supplement
*Recombinant Human Heregulinβ-1	Peprotech, 100-03	100 µg/ml	10 ng/ml	Growth Factor
*Human Activin A	Thermo Fisher Scientific, PHG9014	100 µg/ml	10 ng/ml	Growth Factor
*Recombinant Human IGF-1	Peprotech, 100-11	100 µg/ml	200 µg/ml	Growth Factor

* All growth factors were added fresh to the media every time before use.

Additional References

1. Chambers, S. M., Fasano, C. A., Papapetrou, E. P., Tomishima, M., Sadelain, M., & Studer, L. (2009). Highly efficient neural conversion of human ES and iPS cells by dual inhibition of SMAD signaling. *Nature Biotechnology*, *27*(3), 275–280. https://doi.org/10.1038/nbt.1529
2. Vogt, J., Traynor, R., & Sapkota, G. P. (2011). The specificities of small molecule inhibitors of the TGFß and BMP pathways. *Cellular Signalling*, *23*(11), 1831–1842. https://doi.org/10.1016/j.cellsig.2011.06.019

Supplemental Figures Legends

Figure S1. (Related to Figure 3, 4, and 5). Transcriptome-based PCA and volcano plot of the differentially expressed genes a) Expression-based Principal Component (PC) analysis shows consistent separation between FTD3 patient astrocytes and isogenic controls. **(b)** Volcano plot of genes with a mean of DESeq2-normalized counts >= 10 differentially expressed between FTD3 patient astrocytes and isogenic controls. Genes with absolute log2FoldChange >= 1 and adjusted p-value <= 0.05 are selected as significantly differentially expressed and colored in blue or red if down- or upregulated, respectively. **c)** Left panel: top 15 significantly enriched KEGG and Reactome pathways identified by functional enrichment analysis of all 1133 significantly differentially expressed genes between FTD3 patient astrocytes and isogenic controls. The gene ratio corresponds to the ratio between the differentially expressed and all expressed genes annotated with a term. For each enriched term, the size of the dot indicates the number of differentially expressed genes annotated with this term, while the color shows the FDR of the enrichment test. Right panel: The same representation as in (c) is used for the top 15 significantly enriched Gene Ontology (GO) functions.

Figure S2. (Related to Figure 2). Characterization of autophagy changes at different rapamycin treatment concentrations. a, d, g) Representative ICC images of LC3B and S100β co-labelling of astrocytes at 0nM, 200nM and 500nM rapamycin

treatment. LC3B is a marker of autophagosomes, and FTD3 astrocytes show an increase in LC3 labelling. S100B is utilized as a cytoplasmic marker to visualize LC3B intracellular distribution. Rapamycin treatment does not decrease LC3 labelling. Scale bar: 25 µm. **b-c, e-f, h-i)** LC3B puncta number quantification of FTD3 astrocytes and controls at different rapamycin concentrations. The rapamycin treatments do not rescue LC3B increased markers in FTD3 lines to control line levels. Unpaired student's t-test was performed, standard error of the mean (SEM) is presented and significant differences are indicated by *$p < 0.05$ and **$p < 0.01$. N=3, n=3, N indicates the number of experimental repetitions; n indicates the number of technical replicates per cell line.

Figure S3. (Related to Figure 2). Characterization of autophagy changes at 500 nM rapamycin treatment. **a)** Representative ICC images of P62, AQP4 and RAB7 co-labelling of astrocytes with 500 nM rapamycin treatment. Scale bar: 25 µm. **b)** Representative ICC images of P62, AQP4 and LAMP1 co-labelling of astrocytes 500 nM rapamycin treatment. Scale bar: 25 µm. **c-j)** ICC quantifications of P62, RAB7, and P62 mean area and colocalization puncta number of FTD3 astrocytes and controls. Quantifications demonstrate a FTD3 dependent increase in P62 labeling, P62 mean area of distribution, RAB7 markers and P62-RAB7 co-labelling, which cannot be rescued with rapamycin treatment. **k-n)** LAMP1 and colocalization puncta number quantification of FTD3 astrocytes and controls with 500 nM rapamycin treatment. Quantifications demonstrate an up-regulation in LAMP1 and LAMP1-P62 colocalization in heterozygous FTD3 patient lines compared to controls indicating an increase in autophagic vesicle fusion. In contrast, a significant down-regulation in LAMP1 puncta numbers and LAMP1-P62 colocalization is shown in the homozygous induced CHMP2B astrocyte line compared to the wildtype control demonstrating a decrease in autophagosome-lysosome fusion. Unpaired student's t-test was performed, standard

error of the mean (SEM) is presented and significant differences are indicated by *p < 0.05 and **p <0.01. N=3, n=3, N indicates the number of experimental repetitions; n indicates the number of technical replicates per cell line.

Figure S4. (Related to Figure 2). Ultrastructural analysis of autophagosome accumulations visualized with transmission electron microscopy (TEM). Representative TEM images of 10 weeks mature FTD3 heterozygous lines compared to respective controls. FTD3 astrocyte lines demonstrate an increase in electron-dense cargo-filled vesicles. **a)** Control no rapamycin treatment **b)** 500nM rapamycin treatment. Examples of autophagosomes and autolysosomes are indicated with black arrows. (A= autophagosomes and L= autolysosomes. N=3, n=3, N indicates the number of experimental repetitions; n indicates the number of technical replicates per cell line.

Figure S5. Off-Target effect on CRISPR-Cas9 mediated editing. Representative sequencing chromatographs for wild-type, heterozygous CHMP2B mutation and homozygous CHMP2B mutation are shown with the potential off-target region of the Cas9-gRNA complex underlined in black (bottom).

Figure S6. (Related to Figure 5). Additional Cytokine Profile. a-c) Cytokine Profiling of IL-2, TNF α and IL-1β) of heterozygous FTD3 related astrocyte lines compared to controls. An increase in cytokine secretion is found in astrocyte media from FTD3 patients and heterozygous CHMP2B induced lines compared to controls. Standard error of the mean (SEM) is presented and significant differences are indicated by *p<0.05 and **p<0.01. N=3, n=3, N indicates the number of experimental repetitions; n indicates the number of technical replicates per cell line. **d-e)** Karyogram of heterozygous induced

CHMP2B mutation (left) and homozygous induced CHMP2B mutation (right). Both cell lines expressed normal karyotype profile.